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Does diet drive the evolution of head shape and bite force in chameleons of the genus *Bradypodion*?

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Summary

1. The head is a complex integrated system that is implicated in many vital functions. As such, its morphology is impacted by different and sometimes conflicting demands. Consequently, head shape varies greatly depending on the environment and dietary ecology of an organism. Moreover, given its role in territory defence and mating in lizards, it is also subjected to strong sexual selection in these animals.

2. We investigated the relationships between head shape, bite performance and diet in 14 of the 17 extant *Bradypodion* species to determine whether variation in diet can explain the observed diversity in bite force and head shape in this genus. We also evaluate differences between sexes in terms of the relationships between head shape, bite force and diet and predict tighter relationships in females given that the head in this sex is principally under natural selection.

3. Our results show that there is indeed a correlation between head shape, diet and bite force, but the direction and magnitude are sex-dependent. Whereas we observed a correlation between absolute bite force and head shape in both sexes, size-corrected bite force was correlated with mandible and quadrate shape in females only. Despite strong correlations between bite force and prey hardness, and between prey hardness and head shape, we did not find any relationship between head shape and prey evasiveness.

4. These data suggest that the cranial system in chameleons of the genus *Bradypodion* evolves under natural selection for the ability to eat large or hard prey. Moreover, significant differences in the ecomorphological relationships between the two sexes suggest that sexual selection plays a role in driving the evolution of bite force and head shape. These data suggest that ecomorphological relationships may be sex-dependent.

Key-words: Chamaeleonidae, geometric morphometrics, skull morphology, South Africa

Introduction

The vertebrate cranial system is a complex integrated system involved in different and sometimes conflicting functions including feeding, breathing, drinking and in some cases even locomotion or social display (Cooper & Vitt 1993; Bels, Chardon & Kardong 1994; Schwenk 2000; Herrel, Meyers & Vanhooydonck 2001a; Lappin & Husak 2005). Moreover, it houses the sensory organs and protects the central nervous system (Schwenk 2000; Herrel *et al.* 2007). This makes the skull subject to strong, yet different and potentially conflicting selective pressures. Because of these different functional demands, skull variation in

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vertebrates is enormous (Hanken & Hall 1993). Variation in cranial morphology can be linked to a plethora of factors both intrinsic (e.g. genetic, developmental, anatomical) and extrinsic (e.g. predator–prey interaction, habitat, community structure and dynamics; see Hanken & Hall 1993 for an overview).

Much of the variation in cranial morphology across vertebrates reflects features related to feeding as the skull is important in obtaining, processing and ingesting food and is thus subject to the mechanical requirements of feeding. One of the best known examples reflecting the close link between cranial shape and feeding is the beak of the Darwin's finches (Bowman 1961). Indeed, the beak shows a tight correlation with diet due to the need of the beak to resist feeding-related stresses and strains (Bowman 1961; Soons *et al*. 2010, 2015). Moreover, not only beak shape and size but also skull shape and size vary in birds, leading to differences in lever mechanics and bite force in response to the mechanical demands imposed by diet (Beecher 1962; Herrel *et al*. 2005a,b). The mammalian masticatory apparatus also closely tracks diet in many species. This is reflected in dental morphology (Rensberger 1973; Kay 1975; Santana, Strait & Dumont 2011), cranial and jaw shape, and the size, shape and physiology of the masticatory muscles (deWolff-Exalto 1951; Maynard Smith & Savage 1959; Radinsky 1985; Herrel *et al*. 2008b; Nogueira, Peracchi & Monteiro 2009; Dumont *et al*. 2012).

Lizards are an interesting group for study as their head shape is not only linked to diet (e.g. Herrel *et al*. 2008a,b; Edwards *et al*. 2013) but also to habitat structure (e.g. Mosauer 1932; Bickel & Losos 2002; Goodman & Isaac 2008; Edwards *et al*. 2012; da Silva *et al*. 2014a), competition (Langkilde 2009) and foraging strategy (Verwaijen & Van Damme 2007). Several studies have suggested that prey functional properties are the principal drivers of variation in skull morphology in lizards (Herrel *et al*. 2001c; Verwaijen, Van Damme & Herrel 2002; Measey *et al*. 2011; Edwards *et al*. 2013). Indeed, species consuming hard or large prey typically have wider or taller heads that can accommodate larger jaw muscles (Herrel *et al*. 2001c; Measey *et al*. 2011; Edwards *et al*. 2013; Wittorski, Losos & Herrel 2016; da Silva *et al*. 2016) or allow a more vertical insertion of these muscles (Herrel, Aerts & De Vree 1998), resulting in an increased bite force. Although these relationships may seem intuitive at first sight, diet is not the only selective pressure acting on cranial shape and selection for other traits that may trade off with bite force generating capacity may exist. An excellent example is the trade-off between jaw velocity during song production and bite force in Darwin's finches (Podos 2001; Herrel *et al*. 2009).

Chameleons use their ballistic tongues to capture elusive prey (Bell 1989; Wainwright & Bennett 1992a,b; Herrel *et al*. 2000), and as such, head shape is likely not under selection for fast jaw closing and could rather be optimized for bite force generation allowing them to crush hard or large prey. Moreover, chameleons do not fall into the classical feeding strategies (i.e. 'sit-and-wait' or 'active forager') but are considered cruise foragers, an intermediate strategy between sit-and-wait and active foraging (Butler 2005; Measey, Raselimanana & Herrel 2014). Therefore, chameleons may show different relationships between bite force, head shape and diet compared to other lizards. Of the 11 existing chameleon genera, the genus *Bradypodion* has the potential to show extreme morphological variation as each species has a narrow range, but the genus is found across a wide diversity of habitats (e.g. fynbos, Karoo, succulent Karoo, grassland, Afrotemperate forest and thicket). Moreover, within some species, populations occupy more than one habitat, resulting in strong ecomorphological variation (Tolley & Burger 2007; Tolley & Menegon 2013). For example, differences in head shape between *Bradypodion pumilum* ecomorphs are correlated with functional properties of the dominant prey in their diet (Measey *et al*. 2011) as has been observed in other lizards (Herrel *et al*. 2001c; Verwaijen, Van Damme & Herrel 2002; Edwards *et al*. 2013). Finally, sexual dimorphism in head size, shape and ornamentation exists in *Bradypodion* (da Silva *et al*. 2014b) as is the case in other lizards (e.g. Braña 1996; Herrel, Van Damme & De Vree 1996; Herrel *et al*. 1999a; Butler & Losos 2002).

Here, we investigate whether head shape correlates with functional properties of prey items across the genus *Bradypodion*. We characterized cranial, mandibular and quadrate shape using a three-dimensional geometric morphometric approach to better describe how variation in skull morphology related to variation in bite force and diet. Because sexual dimorphism is strong in *Bradypodion,* we analyse data for both sexes separately (e.g. Hopkins & Tolley 2011; da Silva & Tolley 2013). We predict that because both male–male interactions and courtship involve biting and head displays in addition to diet (see Stuart-Fox 2014 for a review), patterns will be sex-dependent. Given the importance of the ballistic tongue in capturing prey, we predict that in females correlations will be strongest between diet and cranial morphology as the passage of larger prey into the mouth may necessitate larger heads, mandibles and quadrates. Conversely, we predict that males will show tight correlations between cranial morphology and bite force given that both sexual selections act on the ability of males to bite hard (see also Vanhooydonck *et al*. 2010; Lopez-Darias *et al*. 2015). Indeed, the pronounced casque in males is an honest visual signal in some chameleons (Vanhooydonck *et al*. 2007a). Consequently, correlations between cranial shape and bite force are expected to be strong in *Bradypodion* males.

Materials and methods

FIELD SAMPLING

A total of 452 chameleons belonging to 14 of the 17 described species of *Bradypodion* (Tolley *et al*. 2004, 2006; Table 1) were sampled on field trips during the months of January and

Table 1. Species/ecomorphs with their corresponding habitats, sample size (*N*) and body size (snout-vent length) of *Bradypodion* included in this study. Each ecomorph group is indicated with shading. Some ecomorphs are currently defined as populations of that species, whereas others were previously defined as separate species and the taxonomic names have been upheld. (a) includes species where at least two ecomorphs were included in the present study, whereas (b) includes species where only one ecomorph was included

		N		Snout-vent length (mm)		
Species/ecomorphs	Habitat	Female	Male	Female	Male	Comments
(a)						
caffer	Forest	10	6	57·54 ± 9·92	61·96 ± 1·36	Two ecomorphs known
	Grassland	7	11	42·12 ± 1·96	42·60 ± 2·01	
damaranum	Forest	9	19	58·63 ± 11·88	65·46 ± 9·22	Two ecomorphs known
	Fynbos	5	8	65·23 ± 3·22	45·75 ± 10·36	
melanocephalum	Coastal grassland	16	25	57·02 ± 4·22	48·63 ± 3·89	Two ecomorphs included, but additional
	Inland	33	28	49·01 ± 8·80	48·78 ± 5·95	ones are likely
pumilum	Forest	22	27	58·49 ± 12·43	62·07 ± 13·14	Two ecomorphs known
	Fynbos	20	22	49·25 ± 6·45	48·28 ± 5·76	
thamnobates	Mistbelt forest	56	44	68·35 ± 14·83	60·96 ± 13·37	Two ecomorphs included, but additional
	Afrotemperate forest	17	25	66·34 ± 19·21	60·97 ± 14·83	ones are likely
ventrale	Thicket	19	8	67·47 ± 12·77	64·22 ± 9·22	Three ecomorphs included, but additional
taeniabronchum	Fynbos	18	21	50·82 ± 7·56	50·13 ± 4·74	ones are likely. Two ecomorphs were
sp. *Baviaanskloof*	Fynbos	11	5	63·85 ± 4·51	54·32 ± 5·23	previously described as separate species and the names are retained
(b)						
sp. *Karkloof*	Forest	14	10	48·85 ± 13·68	46·34 ± 12·50	Undescribed species with one known ecomorph
caeruleogula	Forest	2	6	44·53 ± 2·94	46·11 ± 6·52	One ecomorph known
nemorale	Grass	19	4	56·64 ± 9·41	52·84 ± 7·10	Two ecomorphs known, but forest ecomorph not included
kentanicum	Forest	2	11	51·98 ± 10·52	51·02 ± 5·35	Two ecomorphs known, but grassland ecomorph not included
occidentale	Succulent Karoo	9	1	86·58 ± 4·87	71·98	One ecomorph known
setaroi	Forest	15	7	58·33 ± 4·10	50·99 ± 3·20	One ecomorph known
transvaalense	Forest	10	12	66·14 ± 12·20	60·26 ± 10·24	One ecomorph known, but others may exist

February between 2009 and 2013. Within some of these species, ecomorphs have been previously recognized (Measey, Hopkins & Tolley 2009; da Silva & Tolley 2013, 2013) and some are included in our data set (Table 1). Animals were brought back to the laboratory measured, tested for bite force and stomach flushed, and then released at the exact site of capture (da Silva *et al*. 2016). All experiments (performance measurements, measurements of snout-vent length and stomach flushing) were conducted within 24 h of capture.

DIET ANALYSIS

Each animal was stomach flushed using a 500-ml syringe and a modified needle with a 30° bend and a two-mm-diameter ball tip with aperture at its apex (Herrel *et al*. 2006; Measey *et al*. 2011). The contents were retrieved in a sieve with a mesh size of 0·5 mm. Stomach contents were transferred and conserved in labelled vials with 70% ethanol. Across all the chameleons sampled, only two male *Bradypodion thamnobates* had empty stomachs. Stomach contents were identified to the lowest possible taxonomic rank using Picker, Griffith & Weaving (2002). Each taxonomic group was then classified according to their functional properties in terms of hardness and evasiveness. Hardness classes (hard, intermediate and soft) are based on the actual forces needed to crush various items (Herrel, Van Damme & De Vree 1996; Andrews & Bertram 1997; Herrel, Verstappen & De Vree 1999b; Herrel *et al*. 1999a, 2001c; Herrel, De Grauw & Lemos-Espinal 2001b; Aguirre *et al*. 2003; Vanhooydonck, Herrel & Van Damme 2007b; Measey *et al*. 2011; Carne & Measey 2013). Prey were also classified in terms of evasiveness (evasive, intermediate and slow) according to Vanhooydonck, Herrel & Van Damme (2007b), Measey *et al*. (2011) and Carne & Measey (2013) (see Table 2). Proportions in both number and mass for each functional prey category (soft, intermediate, hard, slow, intermediate, evasive and all mixed categories such as soft and slow, or soft and evasive) were calculated (Table S1, Supporting Information). Proportions were averaged by species and sex, and a relative importance index (IRI) was calculated for each category (Wyckmans *et al*. 2007) as follows:

$$\mathrm{IRI} = (\%N + \%V) \times \%\mathrm{OC},$$

where $\%N$, $\%V$ and %OC are, respectively, the relative numeric abundance of particular prey category (i.e. the number of items of a given type), the volumetric proportion of a given prey category (i.e. the volume of that prey type) and the frequency of occurrence of that prey category (i.e. the proportion of individuals with that prey type in their stomach). Length (L) and width (W) were measured for each prey item. Based on these measurements, the volume of a spheroid was calculated (ellipsoid volume: $= (4/3)\ \pi\ (L/2)\ (W/2)^2$) and the maximum and mean length, width and volume were calculated for each individual (Table 3).

BITE FORCE

Bite force measurements were performed using a Kistler piezoelectric bite force transducer (type 9203; ±500N; Kistler Inc., Winterthur, Switzerland) mounted into a custom-built holder and

Table 2. Functional classification of prey taxa retrieved from the stomach contents

Hardness	Evasiveness	Abbreviation	Order
Hard	Evasive	HE	Hymenoptera
	Intermediate	HIe	Coleoptera
	Sedentary	HSe	Stylommatophora
Intermediate	Evasive	IE	Isopoda
			Odonata
			Orthoptera
	Sedentary	ISe	Plecoptera
			Coleoptera Pupa
			Lepidoptera Pupa
Soft	Evasive	SE	Blattodea
			Collembola
			Diptera
			Ephemeroptera
			Neuroptera
			Orthoptera Nymph
			Diplura
			Thysanura
	Intermediate	SIe	Dermaptera
			Hemiptera
			Mecoptera
			Myriapoda
			Psocoptera
	Sedentary	SSe	Acari
			Aranea
			Diplopoda
			Julida
			Insect Larva
			Coleoptera Larva
			Lepidoptera Larva
			Lepidoptera
			Mantodea
			Oplione
			Phasmatodea

Classifications based on Vanhooydonck *et al.* (2007a), Vanhooydonck, Herrel & Van Damme (2007b) and Measey *et al.* (2011).

connected to a Kistler charge amplifier (type 5058A; see Herrel *et al.* 1999a). Gape angle (±30°) was standardized by varying the distance between plates according to the head size of the animal and bite position was standardized by mounting metal stops on the bite plates. All animals performed bilateral bites at the tip of the jaws. Bite force measurements were repeated five times, and only, the maximum value was used in the subsequent analysis (Table 4). As a significant correlation between size (snout-vent length, SVL) and bite force was detected ($P < 0{\cdot}001$, $R^2 = 0{\cdot}7$), the residuals of this linear regression were extracted and used in our correlation analyses in addition to absolute bite force data.

GEOMETRIC MORPHOMETRICS

Geometric morphometric data were taken on 34 individuals from the collection in the Port Elizabeth Museum (Bayworld) with at least one male and one female for each species/ecomorph included. Most specimens for which data were available were captured during the austral summer corresponding to the period when we collected diet data. The specimens were scanned using absorption X-ray microtomography (XMT; see Dollion *et al.* 2015 for details on scanning parameters and specimen numbers). Automatic and semi-automatic segmentation (thresholding) and surface rendering were performed using AVIZO7.0 (FEI™, Mérignac, France). Surfaces were exported and loaded into the software package 'LANDMARK' (Wiley *et al.* 2005), and 99 landmarks were then placed on each skull (Dollion *et al.* 2015), mandible and quadrate (Table 5, Fig. 1). These landmarks were used to describe the shape of the skull, mandible and quadrate to investigate morphological variation (Bookstein 1991).

Landmark coordinates for the cranium, mandible and quadrate were analysed independently as these structures can move relative to one another. This avoids bias due to positional variation. A general Procrustes analysis (GPA) (Rohlf & Slice 1990) and allometric effects were examined using multivariate analysis of covariance (MANCOVA) on the shape data with species as factor and size as a covariate. No significant allometric effects were observed (cranium: $P = 0{\cdot}80$; mandible: $P = 0{\cdot}81$; quadrate: $P = 0{\cdot}87$). To compare head shapes between individuals, a principal component analysis (PCA) was performed on the Procrustes residuals. Next we plotted the phylogeny in the morphospace using the 'phylomorphospace' function in R (R Core Team 2016) implemented in the 'phytools' library (Revell 2012). To reduce the dimensionality of the data set (Baylac & Friess 2005) for subsequent analyses, we used the minimal number of principal component (PC) axes that explained 70% or more of the variability (Table 6). All analyses were performed in R (v. 2.15.3; R Core Team 2016) using the libraries RMORPH (Baylac 2013), MASS (Venables & Ripley 2002), APE (Paradis 2012) and ADE4 (Dray, Dufour & Chessel 2007).

COMPARATIVE ANALYSES

To take phylogeny into account, independent contrasts (Felsenstein 1985) were calculated for each characterin MESQUITE (v. 2.75; Maddison & Maddison 2011) using a Brownian motion model of evolution. An existing phylogeny of the genus (Tolley *et al.* 2006) was pruned to include only species and ecomorphs for which dietary data, bite force data and/or morphometric data were available. Some ecomorphs not in the original phylogeny were added with zero branch length because these morphs belong to same species (K. Tolley, unpublished data; Table 1). Independent contrasts for species means (Log_{10}-transformed) of bite force, diet variables and the PC axes of the geometric morphometric analysis were computed using the PDAP package in MESQUITE (Midford, Garland & Maddison 2008). Linear regressions were performed between all shape and performance variables, between performance and ecological variables and between shape and ecological variables. All regressions were forced through the origin as required for independent contrast analyses (Garland, Harvey & Ives 1992). Only those variables that were significantly related were extracted and used as input for a multiple linear regression. We then used the standardized partial regression coefficients (β) to evaluate the contribution of each variable.

Results

MORPHOLOGICAL VARIATION WITHIN BRADYPODION

The *Bradypodion* skull varies among species primarily in parietal shape and length (i.e. the size of the casque), skull width and height, temporal fenestra size and snout curvature (Fig. 2; Figs S1–S3). Whereas *Bradypodion taeniabronchum* and *Bradypodion damaranum* are morphologically the most divergent from each other in males, in females the first axis discriminates between *Bradypodion ventrale* and the grassland morph of *Bradypodion caffer*. Males show a greater range of variation compared to females especially at the back of the skull (Fig. S1). The mandible varies mostly in its width and

Table 3. Average prey dimensions for each species and morph separated by sex

	Prey item size					
	Average length (mm)		Average width (mm)		Average volume (mm)	
Species/morph	Female	Male	Female	Male	Female	Male
caeruleogula	3·75 ± 2·63	3·52 ± 2·14	1·57 ± 0·78	1·81 ± 0·94	8·98 ± 15·98	11·12 ± 16·40
caffer (forest)	4·69 ± 2·73	5·10 ± 2·18	1·86 ± 0·93	2·40 ± 1·05	13·63 ± 21·00	23·95 ± 35·06
caffer (grass)	3·80 ± 1·61	4·19 ± 2·65	1·66 ± 0·81	1·58 ± 0·82	8·62 ± 11·16	9·57 ± 12·33
damaranum (forest)	5·03 ± 3·18	4·19 ± 2·60	2·06 ± 0·89	1·97 ± 1·50	21·78 ± 98·89	15·96 ± 21·00
damaranum (fynbos)	7·03 ± 4·91	6·39 ± 7·83	2·33 ± 1·04	2·25 ± 1·25	34·00 ± 49·34	32·76 ± 60·59
kentanicum	5·20 ± 1·76	4·88 ± 1·82	2·23 ± 0·79	1·98 ± 0·84	16·45 ± 12·35	13·87 ± 16·23
melanocephalum (coastal grassland)	4·80 ± 2·27	3·42 ± 2·07	2·17 ± 0·81	1·42 ± 0·78	16·17 ± 16·23	7·00 ± 11·08
melanocephalum (inland)	3·56 ± 2·28	3·60 ± 2·25	1·49 ± 0·65	1·57 ± 0·75	6·63 ± 11·45	8·35 ± 16·20
nemorale	4·54 ± 2·80	3·36 ± 2·11	2·09 ± 0·97	1·58 ± 0·77	17·09 ± 24·44	7·97 ± 12·99
occidentale	7·10 ± 3·70	5·38 ± 2·79	2·54 ± 1·35	1·80 ± 0·78	40·02 ± 58·88	12·87 ± 13·80
pumilum (fynbos)	5·22 ± 3·67	5·36 ± 3·94	1·66 ± 1·11	1·80 ± 0·92	15·36 ± 30·74	15·05 ± 27·21
pumilum (forest)	4·42 ± 2·93	4·67 ± 3·67	1·50 ± 0·82	1·61 ± 0·93	8·30 ± 13·56	12·81 ± 27·13
setaroi	3·48 ± 2·08	4·01 ± 2·54	2·79 ± 11·33	1·76 ± 0·74	19·19 ± 48·15	9·38 ± 13·00
sp. *Karkloof*	4·20 ± 2·21	3·41 ± 2·12	1·82 ± 0·88	1·44 ± 0·60	11·95 ± 15·76	5·49 ± 7·27
thamnobates (mistbelt)	4·96 ± 2·74	4·59 ± 2·35	2·15 ± 1·07	2·06 ± 1·04	20·28 ± 34·26	17·49 ± 26·50)
thamnobates (afrotemperate)	5·95 ± 2·08	5·28 ± 2·18	2·45 ± 0·93	2·35 ± 1·01	24·02 ± 21·87	21·94 ± 23·20
transvaalense	4·32 ± 3·13	3·34 ± 1·34	1·72 ± 0·84	1·58 ± 0·77	12·62 ± 27·68	7·43 ± 15·78
taeniabronchum	3·00 ± 2·58	2·62 ± 2·27	1·27 ± 0·78	1·16 ± 0·63	5·73 ± 12·51	3·56 ± 7·19
ventrale	4·72 ± 3·65	4·46 ± 3·18	1·77 ± 0·79	1·78 ± 0·84	12·64 ± 21·20	13·08 ± 24·04
ventrale Baviaanskloof (fynbos)	6·02 ± 5·83	4·51 ± 2·40	2·17 ± 1·04	1·88 ± 0·57	23·92 ± 40·59	9·98 ± 8·60

Shading separates species with morphs being grouped within species.

Table 4. Species, morphs, numbers of individuals and bite force for males and females

	Number of individuals		Bite force (*N*)	
Species	Female	Male	Female	Male
caeruleogula	3	6	5·62 ± 1·58	6·14 ± 4·68
caffer (forest)	10	6	12·65 ± 5·30	14·78 ± 7·73
caffer (grass)	7	11	3·43 ± 0·67	3·82 ± 0·77
damaranum (forest)	9	19	17·84 ± 10·23	24·01 ± 7·23
damaranum (fynbos)	7	8	8·62 ± 1·45	3·42 ± 2·78
kentanicum	5	11	7·88 ± 3·96	7·88 ± 3·96
melanocephalum (coastal grassland)	15	23	13·88 ± 4·11	10·37 ± 3·11
melanocephalum (inland)	20	19	9·05 ± 5·23	11·77 ± 5·55
nemorale	20	4	9·32 ± 4·68	9·26 ± 4·46
occidentale	9	1	33·90 ± 6·64	21·71
pumilum (fynbos)	20	22	6·95 ± 2·68	7·50 ± 3·24
pumilum (forest)	23	27	12·29 ± 5·63	16·09 ± 7·48
setaroi	15	7	11·98 ± 1·97	10·93 ± 2·31
sp. *Karkloof*	14	10	9·28 ± 7·99	5·46 ± 2·08
thamnobates (mistbelt)	25	20	24·74 ± 16·35	23·57 ± 17·89
thamnobates (afrotemperate)	12	13	34·25 ± 13·04	30·4 ± 7·58
transvaalense	10	12	17·45 ± 6·11	16·02 ± 6·74
ventrale	19	8	12·66 ± 6·45	9·46 ± 3·82
taeniabronchum	27	32	3·28 ± 1·44	3·65 ± 1·48
ventrale Baviaanskloof (fynbos)	11	7	9·60 ± 1·93	6·48 ± 2·03

Shading separates species with morphs being grouped within species.

length, the shape and size of the coronoid process and the size of the symphysis (Fig. 3). In males, the first axis discriminates between animals with wide and short mandibles like *B. taeniabronchum* and animals with ore elongate narrow mandibles like *Bradypodion melanocephalon*. In females, similar shape variation is detected, yet in this case discriminating between the fynbos morph of *B. pumilum* and the grassland morph of *B. caffer* (Fig. 3). In both sexes, the ventral side of the quadrate articulating with the mandible shows the greatest variability. Whereas in males, the PCA discriminates between *Bradypodion kentanicum* and the forest morph of *B. caffer* on the first axis, and in

Table 5. Definitions of the landmarks placed on the mandible and quadrate

Landmark N°	Description	Side
Mandible		
1	Landmark on the dorsal part of mandibular symphysis	Median
2	Landmark on the ventral part of mandibular symphysis	
3	Landmark anterior at the basis of the coronoid process	Left
4	Landmark on the top of the coronoid process	
5	Landmark posterior at the basis of the coronoid process	
6	Landmark on the dorsal aspect of the posterior process of the angular	
7	Landmark on the ventral part of the posterior process of the angular	
8	Landmark on the foramen at the lateral side of the mandible	
9	Landmark anterior at the basis of the coronoid process	Right
10	Landmark on the top of the coronoid process	
11	Landmark posterior at the basis of the coronoid process	
12	Landmark on the dorsal part of the posterior process of the angular	
13	Landmark on the ventral part of the posterior process of the angular	
14	Landmark on the foramen at the lateral side of the mandible	
Quadratum		
15	Landmark on the medial-most side of anteromedial process of the quadrate	Left
16	Landmark on the lateral side of the dorsal articulation of the quadrate	
17	Landmark on the medial side of the dorsal articulation of the quadrate	
18	Landmark on the lateral side of the ventral articulation of the quadrate	
19	Landmark on the medial side of the ventral articulation of the quadrate	
20	Landmark on the medial-most side of anteromedial process of the quadrate	Right
21	Landmark on the lateral side of the dorsal articulation of the quadrate	
22	Landmark on the medial side of the dorsal articulation of the quadrate	
23	Landmark on the lateral side of the ventral articulation of the quadrate	
24	Landmark on the medial side of the ventral articulation of the quadrate	

females, *Bradypodion occidentale* is the most different from *B. taeniabronchum* (Fig. 4).

RELATIONSHIPS BETWEEN SHAPE AND BITE FORCE

In both sexes, the axis explaining most of the variability in cranial morphology (cranium PC1) is correlated with absolute bite force (males: $P < 0{\cdot}001$; $R^2 = 0{\cdot}89$, $\beta = -1{\cdot}22$; females: $P < 0{\cdot}005$; $R^2 = 0{\cdot}72$, $\beta = 0{\cdot}35$; Fig. 5). An increase in bite force is associated with a larger casque and temporal fenestra, a narrower and taller skull, and a straighter snout. In males, bite force is not associated with variation in mandibular shape. In females, mandible PC3 is negatively correlated with relative bite force ($P < 0{\cdot}05$; $R^2 = 0{\cdot}65$, $\beta = 0{\cdot}86$). Thus, in females, an increase in relative bite force is associated with a wider mandible, a smaller coronoid process and a posterior mandibular foramen that is positioned more anteriorly. In females, relative bite force is also correlated with changes in quadrate shape (quadrate PC2: $P < 0{\cdot}01$; $R^2 = 0{\cdot}86$; quadrate PC3: $P = 0{\cdot}18$; $R^2 = 0{\cdot}87$; $\beta = -0{\cdot}84$) with a higher relative bite force being correlated with a bigger quadrate that shows a different degree of torsion along its length and an enlarged upper process.

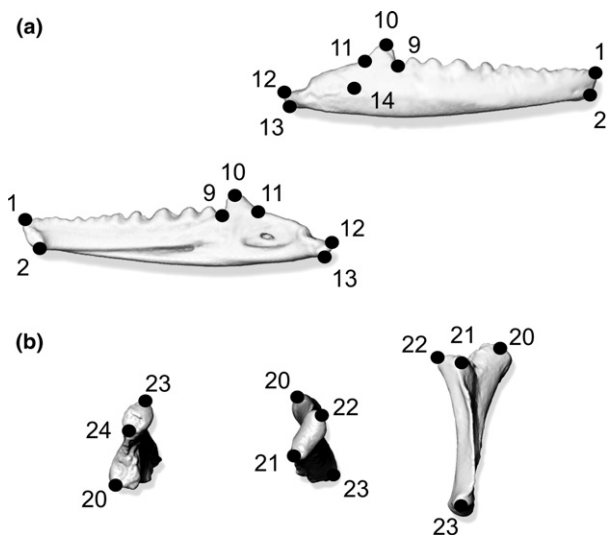

Fig. 1. Landmarks taken on the mandible and quadrate illustrated for *Bradypodion occidentale*. (a) right mandible; top: lateral view, bottom, medial view; (b) right quadrate; left: ventral view, middle: dorsal view, right: lateral view.

RELATIONSHIPS BETWEEN SHAPE AND DIET

No correlations were observed between the principal axis of variation (cranium PC1) in cranial shape and diet in either sex. In males, cranium PC2 is correlated with the relative importance of hard prey in the diet ($P < 0{\cdot}01$; $R^2 = 0{\cdot}46$). An increased consumption of hard prey occurs with a wider cranium, straighter snout, more elevated parietal and squamosal that is more anteriorly positioned. In females, cranium PC2 is correlated with the relative importance of sedentary prey in the diet ($P < 0{\cdot}05$; $R^2 = 0{\cdot}31$). An increase of sedentary prey in the diet is correlated with a straighter and shorter parietal, a less elevated cranium

Table 6. Summary of the principal component analyses performed on the morphometric data

Structure	Principal component	Abbreviation	% variability		Total variability	
			Male	Female	Male	Female
Cranium	1	cranium1	37·67	25·40	79·29	72·14
	2	cranium2	11·80	12·89		
	3	cranium3	10·77	11·38		
	4	cranium4	7·40	8·96		
	5	cranium5	6·38	7·29		
	6	cranium6	5·27	6·22		
Mandibule	1	mandible1	53·21	44·46	78·43	73·00
	2	mandible2	16·76	15·96		
	3	mandible3	8·46	12·58		
Quadrate	1	quadrate1	47·01	44·12	80·46	81·44
	2	quadrate1	18·11	22·69		
	3	quadrate1	15·34	14·63		

roof and a longer snout. Cranium PC3 and PC4 are correlated with mean prey width in females (PC3: $P < 0{\cdot}001$; $R^2 = 0{\cdot}72$; PC4: $P < 0{\cdot}005$; $R^2 = 0{\cdot}51$). An inspection of the associated shapes shows that an increase in mean prey width is associated with a wider skull, a straighter squamosal and a more curved casque.

No correlations were observed between the principal axis of variation (PC1) in mandible shape and diet in females. In contrast, in males, mandible PC1 was found to correlate negatively with the relative proportion of sedentary prey of intermediate hardness ($P < 0{\cdot}01$; $R^2 = 0{\cdot}66$, $\beta = 0{\cdot}12$) and positively with maximal prey length ($P < 0{\cdot}05$; $R^2 = 0{\cdot}66$, $\beta = 0{\cdot}43$). Thus, an increase in the relative importance of sedentary prey of intermediate hardness and a decrease in the maximal prey length are associated with a narrower and longer mandible. In males, mandible PC2 is negatively correlated with the relative importance of soft prey in the diet ($P < 0{\cdot}005$; $R^2 = 0{\cdot}47$), while in females, mandible PC2 is negatively correlated with the relative importance of soft sedentary prey ($P < 0{\cdot}001$; $R^2 = 0{\cdot}68$, $\beta = -0{\cdot}36$) and the relative overall proportion of sedentary prey into the diet ($P < 0{\cdot}005$; $R^2 = 0{\cdot}68$, $\beta = -0{\cdot}57$). Thus, narrower mandibles with a smaller mandibular symphysis are associated with an increase in soft prey in the diet of males, and with an increase in the importance of soft sedentary prey in the diet, and sedentary prey more generally, in females. In males, mandible PC3 is negatively correlated with the relative proportion of sedentary prey ($P < 0{\cdot}005$; $R^2 = 0{\cdot}68$, $\beta = -0{\cdot}39$), as well as maximal prey width ($P < 0{\cdot}1$; $R^2 = 0{\cdot}68$, $\beta = -0{\cdot}35$). In females, mandible PC3 is positively related to the relative proportion of sedentary prey of intermediate hardness ($P < 0{\cdot}05$; $R^2 = 0{\cdot}65$, $\beta = 0{\cdot}46$). In males, an increase of the relative proportion of sedentary prey in the diet, as well as an increase in prey width, goes along with thicker mandibles and a larger coronoid process. In females, an increase in the relative proportion of sedentary prey in the diet is associated with a narrower mandible, a taller coronoid process and a posterior mandibular foramen that is positioned more posteriorly.

In males, quadrate PC1 is negatively correlated with the relative proportion of evasive prey into the diet ($P < 0{\cdot}05$; $R^2 = 0{\cdot}34$). Thus, a decrease in the proportion of evasive prey is observed in animals with a longer anterior part of the upper process of the quadrate and a more inclined upper process. In females, quadrate PC1 is correlated with the mean prey length ($P < 0{\cdot}05$; $R^2 = 0{\cdot}34$). Thus, chameleons eating longer prey have a longer anterior part of the upper process of the quadrate and a more inclined upper process. In both sexes, the mean prey length is correlated with quadrate PC2, albeit negatively in females ($P < 0{\cdot}01$; $R^2 = 0{\cdot}60$, $\beta = 2{\cdot}24$) and positively in males ($P < 0{\cdot}05$; $R^2 = 0{\cdot}30$). In addition, quadrate PC2 is positively related to the relative proportion of evasive prey of intermediate hardness in females ($P = 0{\cdot}22$; $R^2 = 0{\cdot}60$, $\beta = 0{\cdot}53$). Consequently, a slightly smaller quadrate is correlated with an increase of the average prey length in both sexes; a bigger quadrate is observed when the proportion of prey with intermediate hardness in the diet increases. Finally, quadrate PC3 is correlated with the relative importance of hard sedentary prey in both sexes (females: $P = 0{\cdot}012$; $R^2 = 0{\cdot}87$; $\beta = 0{\cdot}002$; males: $P = 0{\cdot}019$, $R^2 = 0{\cdot}38$; $\beta = 0{\cdot}35$). In both sexes, the increase of the proportion of hard sedentary prey in the diet is observed in chameleons with a bigger upper process of the quadrate. In males, quadrate PC3 is also negatively correlated with the relative importance of evasive prey of intermediate hardness ($P = 0{\cdot}018$; $R^2 = 0{\cdot}38$; $\beta = 1{\cdot}68$). Thus, chameleons with a smaller upper quadrate process eat fewer evasive prey of intermediate hardness. In females, quadrate PC3 is positively associated with mean prey width ($P = 0{\cdot}024$; $R^2 = 0{\cdot}87$; $\beta = 0{\cdot}059$) and negatively with the relative importance of sedentary prey of intermediate hardness in the diet ($P < 0{\cdot}05$; $R^2 = 0{\cdot}87$; $\beta = 0{\cdot}54$). Thus, in females, a larger upper quadrate process is observed when the proportion of sedentary prey of intermediate evasiveness increases in the diet and when the average prey width increases.

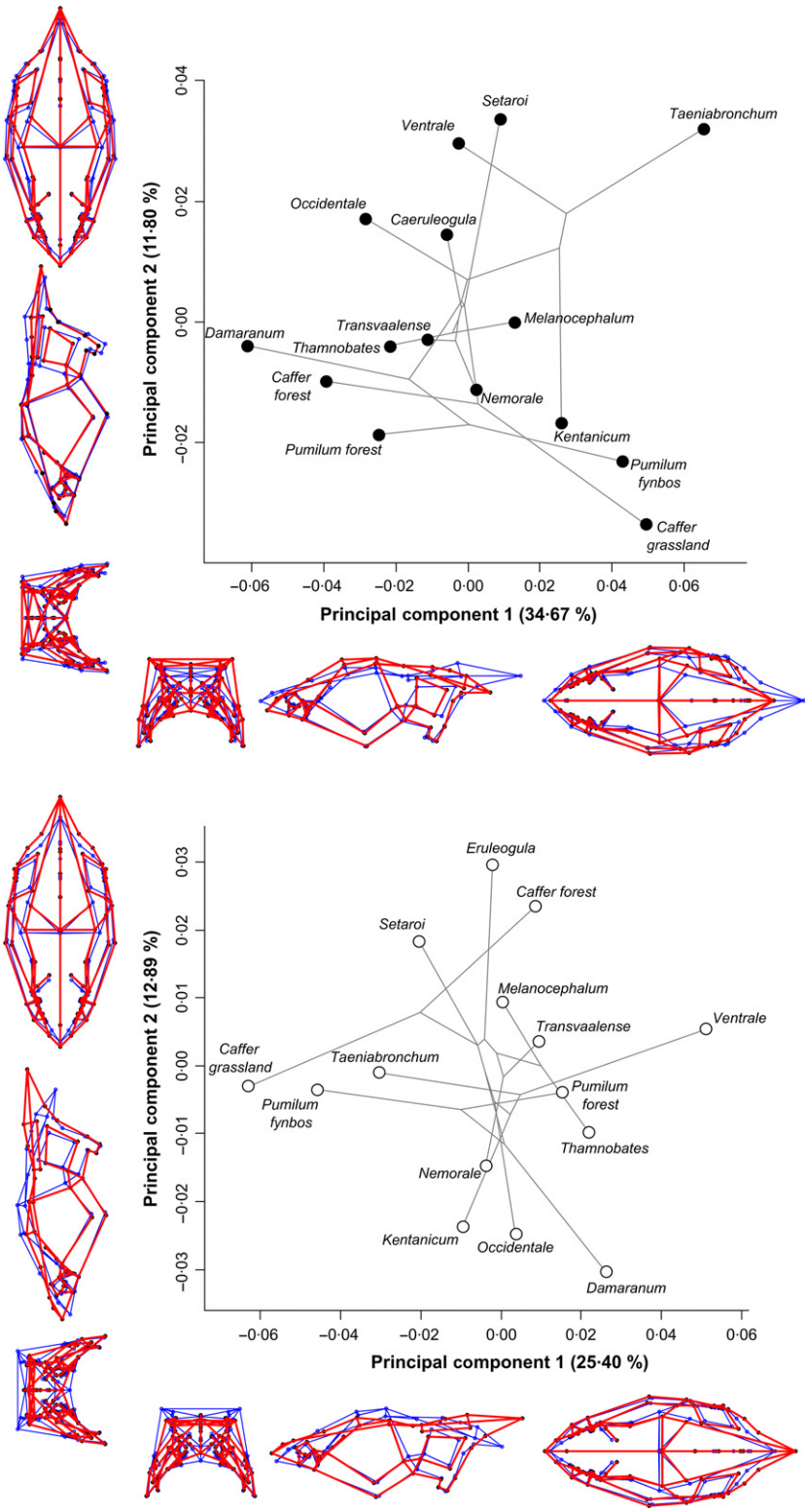

Fig. 2. Phylomorphospaces representing the shape variation of the cranium in the genus *Bradypodion* for males (top) and females (bottom). Along the axis are represented the visualizations of the variation corresponding to the two first axis of the principal component analysis (PCA) (PC1 and PC2). Red shapes illustrate the positive side of the axis; blue ones the negative side.

RELATIONSHIPS BETWEEN BITE FORCE AND DIET

Absolute bite force is correlated with the maximal prey volume consumed in males only ($P < 0{\cdot}001$; $R^2 = 0{\cdot}69$). In females, bite force is strongly correlated with the mean width of the prey eaten ($P < 0{\cdot}001$; $R^2 = 0{\cdot}76$). In males, relative bite force (i.e. independent of body size) is associated with the relative importance of hard prey in the diet ($P = 0{\cdot}040$; $R^2 = 0{\cdot}59$) as well as the relative importance of hard sedentary prey in the diet ($P = 0{\cdot}02$; $R^2 = 0{\cdot}59$). In females, relative bite force is correlated with the relative proportion of hard and intermediately evasive prey in the diet ($P < 0{\cdot}01$; $R^2 = 0{\cdot}86$).

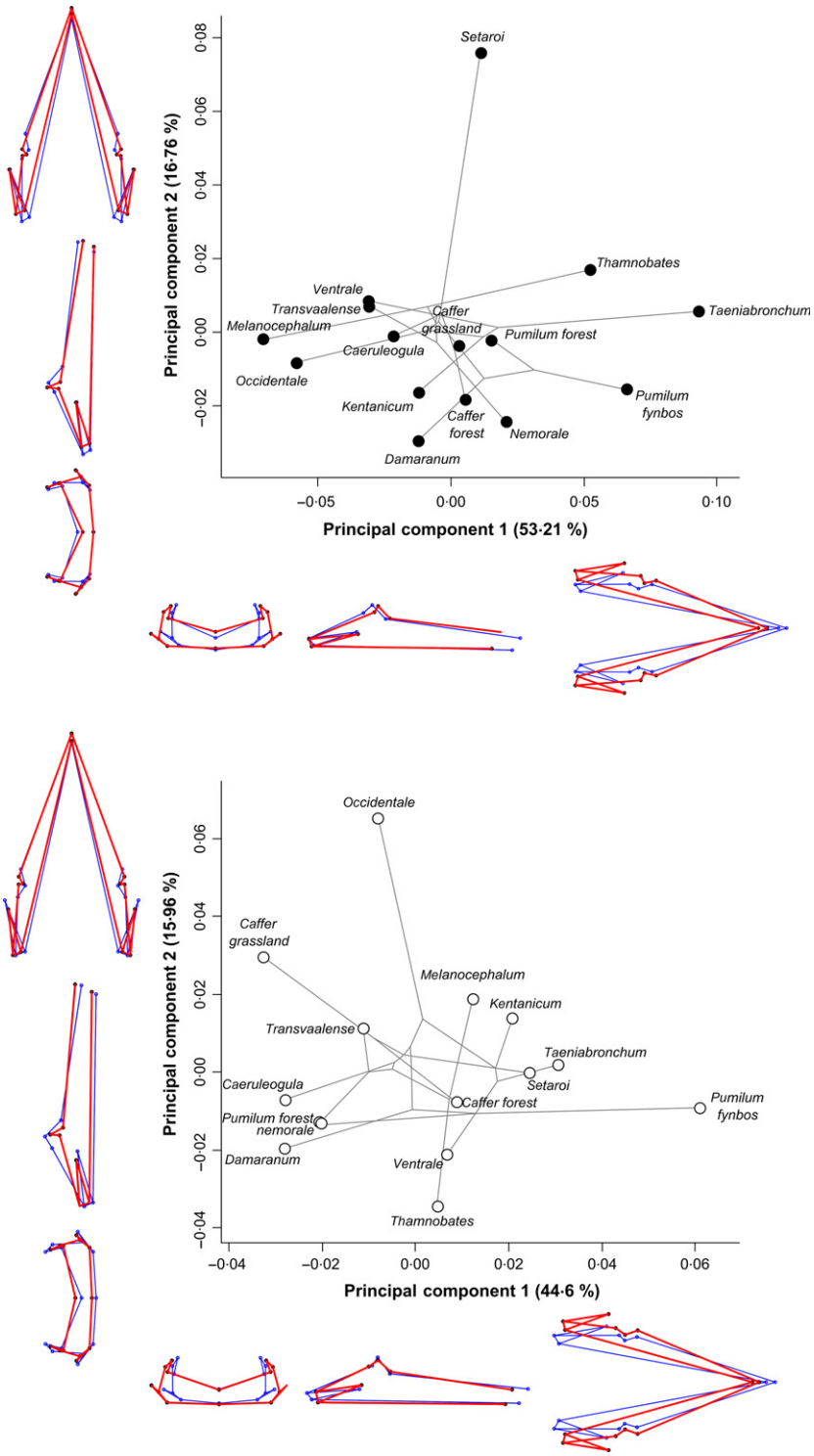

Fig. 3. Phylomorphospaces representing the shape variation of the mandible in the genus *Bradypodion* for males (top) and females (bottom). Along the axis are represented the visualizations of the variation corresponding to the two first axis of the principal component analysis (PCA) (PC1 and PC2). Red shapes illustrate the positive side of the axis; blue ones the negative side.

Discussion

As predicted, there is a significant link between skull shape and bite force in *Bradypodion*. The principal determinants of bite force were parietal shape (= casque shape), cranium height and width, and snout curvature. We found a positive correlation between the casque size and bite force across the entire genus, similar to that found for *B. pumilum* (Measey, Hopkins & Tolley 2009). Moreover, longer and more convex snouts are associated with higher bite forces. An increase in casque and temporal fenestra size increases the space available for the *adductor mandibulae externus* and the *pseudotemporalis* muscles, likely responsible for the higher bite force. However, changes in snout length and shape are more complex to understand but could be related to the stresses and strains induced by biting on the maxilla, with more convex snouts potentially being better at resisting these stresses. However, this

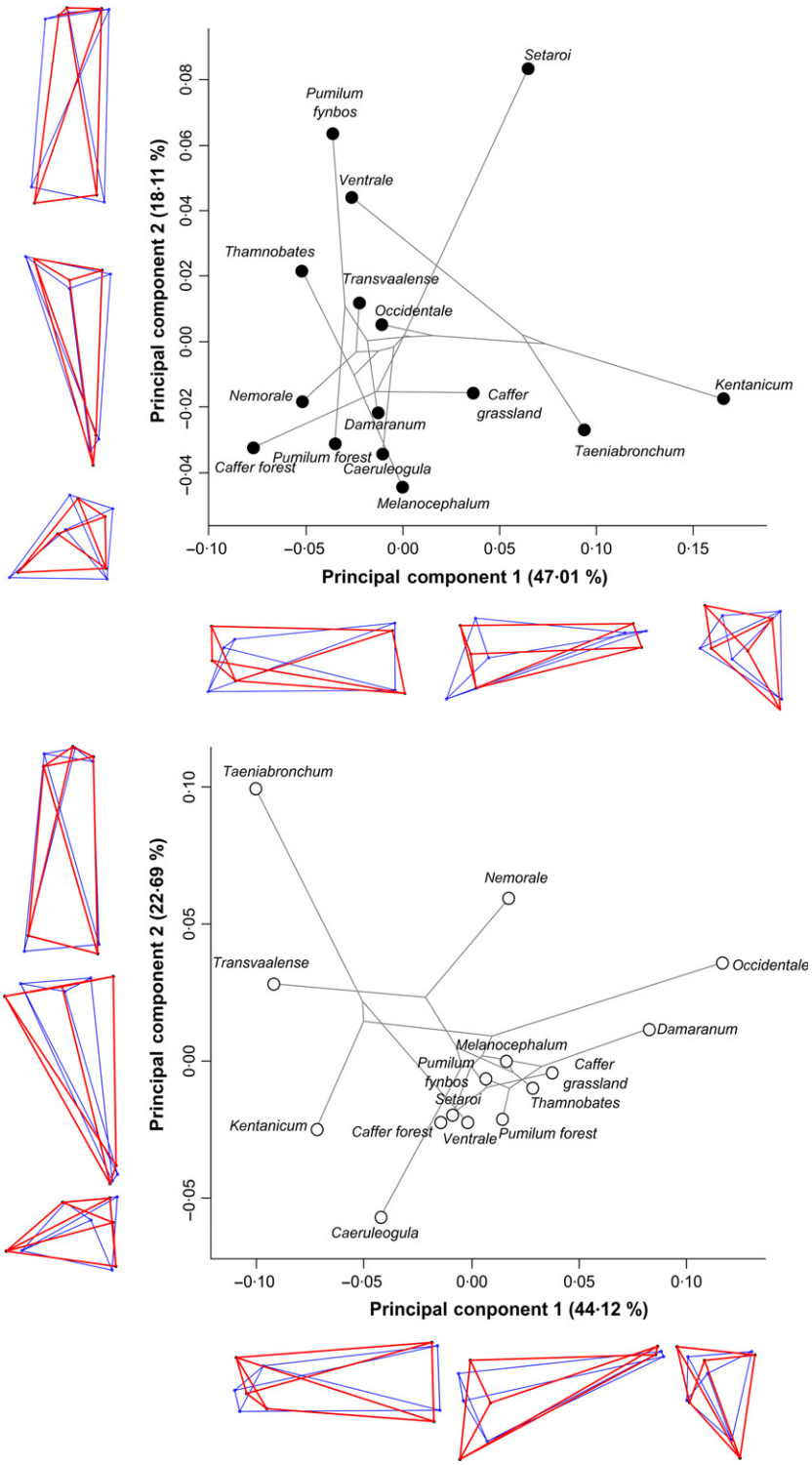

Fig. 4. Phylomorphospaces representing the shape variation in the quadrate in the genus *Bradypodion* for males (top) and females (bottom). Along the axis are represented the visualizations of the variation corresponding to the two first axis of the principal component analysis (PCA) (PC1 and PC2). Red shapes illustrate the positive side of the axis; blue ones the negative side.

remains to be tested using finite element analyses or similar approaches (Rayfield 2007). Bite force was also strongly correlated with mandible and quadrate shape in females but not males. This was unexpected given the importance of the mandible in providing the insertion of the jaw muscles. Possibly, our shape quantification did not accurately capture the variation in the coronoid and the lateral side of the mandible, known to be important insertion sites for the jaw adductors (Fabre *et al.* 2014a,b). Three-dimensional geometric morphometric approaches using sliding landmarks may be better suited to capture subtle shape variation (Cornette *et al.* 2013; Cornette, Tresset & Herrel 2015) and may potentially change our interpretation of these patterns.

Variation in mandible shape was significantly associated with variation in diet in both males and females.

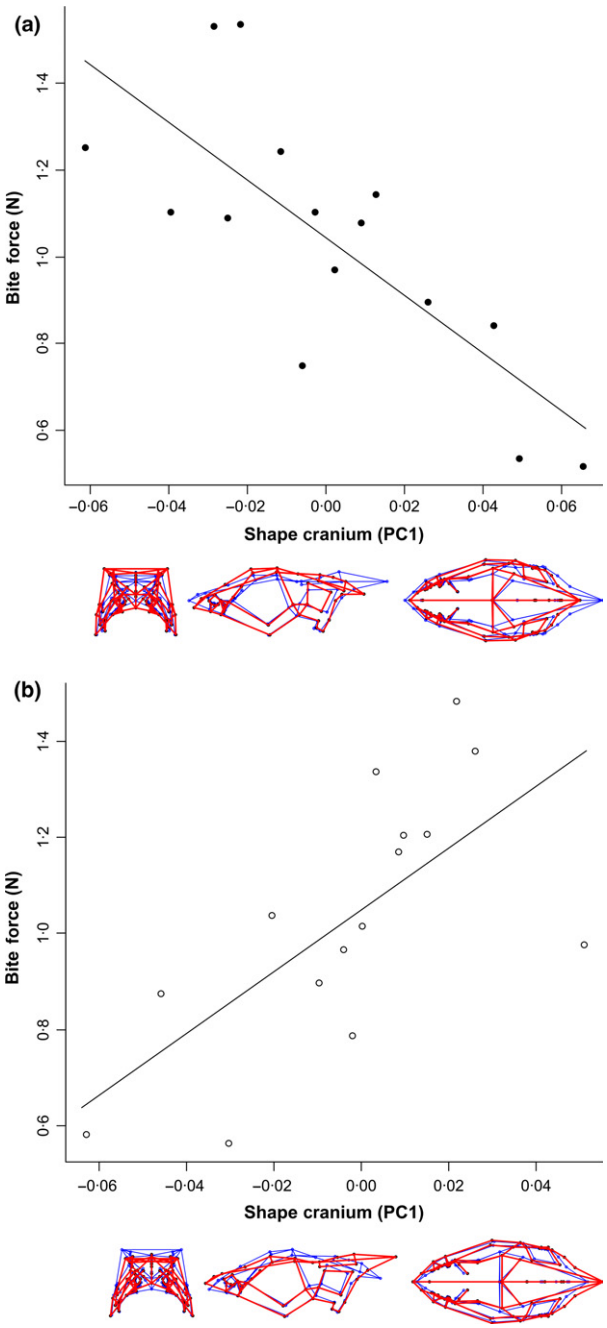

Fig. 5. Linear regression of the Log_{10}-transformed absolute bite force against the first axis of cranium shape for (a) males and (b) females. The shapes associated with variation in bite force are visualized (red for the positive part of the axis, blue for the negative part of the axis). Despite the opposite trend of the regression line, patterns are similar in males and females with animals with larger casques having higher bite forces. Note that in contrast to the raw data depicted in the figure, the analyses were performed on the independent contrasts of the data to take into account the phylogenetic history of the species.

Specifically, species with narrow and long mandibles had a greater proportion of soft and sedentary prey in both males and females. This makes intuitive sense as soft, sedentary prey do not require robust mandibles that can resist larger forces. Variation in the shape of the coronoid, the principal insertion site for the jaw adductors in lizards (e.g. Schwenk 2000; Wittorski, Losos & Herrel 2016), was also associated with variation in diet, but differently so in males and females. Whereas in males taller coronoids and thicker mandibles were associated with a greater proportion of wider sedentary prey, in females a taller coronoid, yet more slender mandible, was associated with a greater proportion of sedentary prey in the diet. Variation in the quadrate was also significantly associated with variation in diet. Most of the ecologically relevant variation was situated in the side of the quadrate that articulates with the squamosal and the rest of the skull. Although the quadrate in chameleons has been suggested to be hyperstreptostylic and thus allowing significant movement at the quadrato-squamosal joint (Iordansky 1996), no *in vivo* data on quadrate mobility exist rendering the functional interpretation of shape variation difficult. In *Bradypodion,* harder prey are associated with a more robust quadrato-squamosal articulation, possibly to reinforce the joint when biting on harder prey. Other shape changes observed in relation to diet are more subtle but harder to interpret functionally. For example, the longer upper process associated with a reduction of the proportion of evasive prey into the diet may be related more to changes in the overall insertion area for the m. adductor posterior (which takes its origin on the anterior and dorsal face of the quadrate) than with changes in quadrate stability or mobility. However, the paucity of functional studies that have quantified possible quadrate mobility *in vivo* prevents us from providing further functional hypotheses.

Bite force was in turn linked to prey size, particularly to the volume of the prey. This is likely due to the known positive correlation between prey size and its hardness (Herrel, Van Damme & De Vree 1996; Herrel *et al.* 1999b; Herrel, Meyers & Vanhooydonck 2001a; Aguirre *et al.* 2003). However, when bite force is corrected for overall variation in chameleon body size, it is no longer correlated with prey size, but rather to prey hardness. Unexpectedly, relative bite force is negatively correlated with the overall importance of hard prey in the diet, yet positively correlated with the importance of hard sedentary prey in the diet of males specifically. Relative bite force was also associated to the importance of hard prey of intermediate mobility in females. When exploring the types of prey eaten, it becomes apparent that several hard prey types were also small (e.g. *Hymenoptera* and *Coleoptera*). The exceptions are the *Stylommatophora* (land snails) which are large and sedentary, and indeed, relative bite force is positively correlated with the presence of these types of prey in the diet. Although this suggests that the use of prey functional classifications can render the interpretation of the results complex, our results do show unequivocally that the evolution in head shape and bite force is linked to diet in this chameleon genus. More specifically, the size and hardness of the prey eaten appear to impose constraints on bite force which, in turn, appear to drive the evolution of cranial shape.

We also found different patterns in ecomorphological variation between sexes, suggesting that the selective pressures acting on the two sexes are different. In males, for example, there is no relationship between relative bite force and head shape indicating that the morphology of the cranium in males is evolving due to selection on absolute bite force. This might be related to the fact that bite force in males is not only under natural selection for the consumption of hard prey, but also under strong sexual selection (da Silva *et al*. 2014b). Chameleons, like many other lizards (e.g. Lailvaux *et al*. 2004; Huyghe *et al*. 2005), will bite one another during intraspecific conflicts. However, before contests escalate into actual fights, chameleons will use visual signals to assess their opponent (Stuart-Fox, Moussalli & Whiting 2007; Stuart-Fox & Moussalli 2008). Consequently, body size and absolute casque size are likely important traits for signalling the fighting ability of male chameleons. This suggests that selection may be acting on body and head size in addition to performance, which may explain the observed lack of correlations between relative bite force and head shape.

Although our results show strong links between bite force and diet, data regarding prey availability will be important to determine whether *Bradypodion* are selecting prey based on their physical ability to crush prey of different hardness. Stomach contents may, alternatively, reflect the diversity and abundance of prey in the habitat more than predator selectivity. However, Carne & Measey (2013) showed that chameleons of the genus *Bradypodion* actively avoided hard prey in summer, yet included these prey in winter when prey availability was decreased. Despite this, we cannot rule out that the relation between skull morphology and diet may simply reflect differences in prey availability in the different habitats (Halaj, Ross & Moldenke 2000). In the case of *Bradypodion,* this is a possibility as habitat structure may drive head shape, and as such impose limits on bite force capacity (Stuart-Fox & Moussalli 2007; Measey, Hopkins & Tolley 2009; da Silva & Tolley 2013; da Silva *et al*. 2014a,b). For example, the forest and fynbos morphs of *B. pumilum* had very differently shaped heads, mandibles and quadrates (Figs 2–4). Similarly, the forest and grassland morphs of *B. caffer* also displayed very different cranial shapes that surpassed variation between species in some cases. It would be of interest to test the direct effect that the environment may impose on head shape evolution in chameleons (Hopkins & Tolley 2011; da Silva & Tolley 2013). Moreover, it would be of interest to evaluate possible covariation between body and head shape in these animals. As body shape in female chameleons is strongly constrained by reproductive output (see review in Measey, Raselimanana & Herrel 2014), part of the variation in head width, for example, may simply be due to overall selection on wide and large abdomen size in females.

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Data accessibility

Data are deposited Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5bb65 (Dollion *et al*. 2016).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Scatterplot illustrating the results of principal components analyses performed for the (a) the cranium, (b) the mandible and (c) the quadrate for males and female *Bradypodion*.

Fig. S2. The shape variation of the cranium in female *Bradypodion* as summarized by the first six principal components.

Fig. S3. The shape variation of the cranium in male *Bradypodion* as summarized by the first six principal components.

Fig. S4. The shape variation of the mandible in male (top) and female (bottom) *Bradypodion* as summarized by the first three principal components.

Fig. S5. The shape variation of the quadrate in male (top) and female (bottom) *Bradypodion* as summarized by the first three principal components.

Table S1. Relative importance index of prey items retrieved from the stomachs of the different species and morphs included in the analysis.